

N 9 0 - 2 6 4 7 0

EXPERIMENT K-6-17

**STRUCTURAL CHANGES AND CELL TURNOVER IN THE RAT'S SMALL INTESTINE
INDUCED BY SPACEFLIGHT**

Principal Investigator:

**R.W. Phillips
College of Veterinary Medicine and Biomedical Sciences
Colorado State University
Fort Collins, Colorado 80523**

Co-Investigators:

**H. R. Sawyer
Colorado State University
Fort Collins, Colorado 80523**

**K. V. Smirnov
Institute of Biomedical Problems
Moscow, USSR**

INTRODUCTION

The purpose of this project was to test the hypothesis that the generalized, whole body decrease in synthetic activity associated with microgravity conditions of space flight as evidenced by negative nitrogen balance and muscle atrophy (Nicogossian and Parker, 1982; Oganov, 1981), as well as inhibited lymphocyte proliferation (Bechler and Cogoli, 1986), would be evident in cells characterized by a rapid rate of turnover. As a model we chose to study the turnover of mucosal cells lining the jejunum of the small intestine, since these cells are among the most rapidly proliferating in the body. Under normal conditions, epithelial cells that line the small intestine are continually produced in the crypts of Lieberkuhn. These cells migrate out of the crypts onto intestinal villi, are progressively "pushed up" the villus as new crypt cells are formed, and ultimately reach the tip of villi where they are then desquamated. In rats, the entire process, from initial proliferation in crypts to desquamation, takes approximately 2 days (Cairnie et al., 1965; Lipkin, 1973). In this study, we determined the mitotic index for mucosal cells lining the proximal, middle, and distal regions of the jejunum in rats from three treatment groups (synchronous control, vivarium control and flight), and measured the depth of the crypts of Lieberkuhn and the length of villi present in each of the three jejunal regions sampled.

MATERIALS AND METHODS

Tissue Samples

Tissue samples (1 cm in length) representing the proximal, middle and distal regions of the jejunum from each of 5 rats from the synchronous, vivarium and flight groups were processed and shipped to Colorado State University per pre- and post-flight protocols described for Cosmos 1887 Experiment K-6-17. Briefly, jejunal regions of interest were removed and flushed with 1 to 2 ml of physiological saline. Immediately thereafter each sample was flushed with 2 ml of a solution of 4% glutaraldehyde-0.1M cacodylate (pH 7.4) containing 5% sucrose and placed into 25 ml screw-top vials containing approximately 20 mls of the same fixative. After 6 - 24 hr of fixation, the samples were rinsed 3 times in 0.1 M cacodylate buffer and shipped to Colorado State University. Upon arrival, each sample was cut into 4 equal segments, post-fixed in 1% osmium tetroxide for 90 min, washed in cacodylate buffer, dehydrated in a graded series of ethanols and embedded in Polybed 812.

Mitotic Index

Sections 1 μ m-thick were cut from each of the four segments from each of the three jejunal regions per animal and stained with toluidine blue. To accurately determine the mitotic index for each region, at least 2000 cells per region per animal were examined. Since mitosis is restricted to the crypts of Lieberkuhn (Lipkin, 1973), cells outside the crypts proper were not considered in determining mitotic indices. Prior to evaluation all slides were coded so that the technician reading the slides did not know the region or treatment group being examined.

Villus Length and Crypt Depth

To determine villus length and crypt depth at least 25 villi and crypts were measured per region per animal. Measurements were obtained using a computerized image analysis system coupled to a bright field microscope equipped with a 40X objective and a video camera. Special care was taken to ensure that measurements were taken only on villi and crypts that had been cut in cross-section.

Statistics

All data were statistically analyzed by analysis of variance and differences between means were detected using the Student-Newman-Keuls procedure.



RESULTS

Data obtained regarding the mitotic indices for the three jejunal regions in each treatment group (i.e., synchronous, vivarium and flight) are summarized in Table 1. In the vivarium group the number of mitotic figures was consistently lower in the middle and distal jejunal regions when compared to the same regions in the synchronous control and flight groups. In the proximal jejunum the flight group had more mitotic figures than either of the control groups. There was no significant variation in the mitotic indices among the three different anatomical regions examined in the animals included in the vivarium and flight groups. In contrast, animals in the synchronous control group had more mitotic figures in the middle jejunum region.

When the data were analyzed by jejunal region among treatment groups several differences were noted (Table 1). The number of mitotic figures observed in the proximal jejunum of the flight animals was higher compared to either the synchronous or vivarium animals. Conversely, in the middle and distal jejunum, both the synchronous controls and flight animals had an increased number of mitotic figures compared to vivarium.

As summarized in Table 2, the height of jejunal villi in flight animals was not significantly different from that observed in animals included in the synchronous and vivarium groups. Although there was a progressive and significant decrease in the height of villi from the proximal to distal region of the jejunum in both the flight and synchronous animals, no significant differences were detected among the jejunal regions examined in animals included in the vivarium group. This was due to the high degree of variability in measurements obtained for the respective jejunal regions in the vivarium group.

Irrespective of treatment group, the depth of crypts tended to be greater in the proximal jejunal region than in the middle or distal regions (Table 3). However, statistically significant differences were detected only in the flight and synchronous groups. With respect to region by treatment interactions, the only marked difference was that the crypt depth in the proximal jejunal region in the flight animals was less than that measured in the synchronous animals.

DISCUSSION

Although some statistical differences were noted in number of mitotic figures among treatment groups and jejunal regions examined, no clear pattern of change was evident when flight animals were compared to both synchronous and vivarium control groups. This is not surprising given the length of time from return to earth, and ultimate recovery and tissue collection (i.e. approximately 50 hours). Since the average life-span of intestinal epithelial cells in rats is slightly less than 2 days (Cairnie et al., 1965; Lipkin, 1973), very few, if any, of the cells present in the small intestine at the time of recovery would have been generated during flight.

Since no consistent differences were detected in the mitotic index of jejunal mucosal cells in the flight group compared to the synchronous and vivarium control groups, it is not surprising that no consistent differences were detected in the length of villi or crypt depths. Although some regional differences in villus height and crypt depth were noted, such differences appear to be normal and cannot be attributed to treatment effects.

SUMMARY AND CONCLUSIONS

The mitotic indices, villus heights and crypt depths were determined in each of three jejunal regions (proximal, middle and distal) for five animals each in the flight, vivarium and synchronous groups. Because of the rapid turnover of intestinal mucosal cells, and due to the delay in recovering the flight animals, it is not known if cell turnover is affected by microgravity conditions associated with space flight. However, since there were no consistent differences between animals in the flight group and

those in the synchronous and vivarium control groups, it appears that any effects of microgravity on the turnover of jejunal mucosal cells are short-lived and rapidly return to normal. The data obtained in this study will be valuable as a comparative reference when similar tissues are collected during or more immediately after return from microgravity conditions. Thus, this study represents an initial step in determining the effects of microgravity on the proliferation and turnover of intestinal mucosal cells.

REFERENCES

1. Bechler, B. and A. Cogoli. 1986. Lymphozyten sind schwerkraftempfindlich. *Naturwissenschaften* 73:400-403.
2. Cairnie, A.B., L.F. Lamerton and G.G. Steele. 1965. Cell proliferation studies in the intestinal epithelium of the rat. I. Determination of the kinetic parameters. *Exp. Cell Res.* 39:528-538.
3. Lipkin, M. 1973. Proliferation and differentiation of gastrointestinal cells. *Physiol. Rev.* 53:891-915.
4. Nicogossian, A.E. and J.F. Parker, Jr. 1982. Space physiology and medicine. NASA SP-447. Washington, D.C. National Aeronautics and Space Administration. 324 p
5. Oganov, V.S. 1981. Results of biosatellite studies of gravity-dependent changes in the musculoskeletal system of mammals. *Physiologist* 24 (Suppl.):S55-S58.



TABLE 1. MITOTIC INDEX FOR JEJUNAL MUCOSA CELLS

TREATMENT GROUPS (N=5 ANIMALS/GROUP)

REGIONS	SYNCHRONOUS	VIVARIUM	FLIGHT
PROXIMAL	2.9 \pm 2 ^{A,a}	2.8 \pm 2 ^a	3.7 \pm 2 ^b
MIDDLE	3.9 \pm 3 ^{B,a}	2.7 \pm 1 ^b	3.4 \pm 1 ^a
DISTAL	3.3 \pm 4 ^{A,a}	2.7 \pm 3 ^b	3.2 \pm 2 ^a

*Means (\pm SEM) with different lower case letter superscripts within rows are different ($p < .05$).

**Means (\pm SEM) with different with upper case letter superscripts within columns are different ($p < .05$).

TABLE 2. LENGTH (μ m) OF JEJUNAL VILLI

TREATMENT GROUPS (N=3 ANIMALS/GROUP)

REGIONS	SYNCHRONOUS	VIVARIUM	FLIGHT
PROXIMAL	557 \pm 24 ^A	556 \pm 89	600 \pm 29 ^A
MIDDLE	486 \pm 8 ^B	510 \pm 40	497 \pm 23 ^B
DISTAL	296 \pm 9 ^C	376 \pm 78	317 \pm 17 ^C

*Means (\pm SEM) with different upper case letter superscripts within columns are different ($P < .05$).

TABLE 3. JEJUNAL CRYPT DEPTHS (μ m)

TREATMENT GROUPS (N=5 ANIMALS/GROUP)

REGIONS	SYNCHRONOUS	VIVARIUM	FLIGHT
PROXIMAL	176 \pm 8 ^{A,a}	160 \pm 8 ^b	148 \pm 3 ^{A,b}
MIDDLE	139 \pm 6 ^B	137 \pm 4	128 \pm 2 ^B
DISTAL	120 \pm 3 ^B	141 \pm 10	123 \pm 4 ^B

*Means (\pm SEM) with different lower case letter superscripts within rows are different ($P < .05$).

**Means (\pm SEM) with different upper case letter superscripts within columns are different ($P < .05$).